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Selection of phage-displayed superantigen by binding to cell-surface MHC class II

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Abstract

We have expressed the superantigen staphylococcal enterotoxin A (SEA) on the surface of bacteriophage as a fusion with the gene VIII protein (gVIIIp). This phage-displayed superantigen retains the properties inherent in the natural protein. It binds to MHC class II and activates T-cells bearing appropriate Vβ regions. A flexible 5-amino acid linker sequence between the SEA molecule and the phage coat protein improved the production of functional phage-displayed SEA. Binding to MHC class II-expressing cells effectively selected SEA-phage from non-SEA-phage background. This indicates that this will be an effective method for selecting new specificities of superantigen from libraries of SEA mutants and for cloning of novel superantigens.

Keywords: T lymphocyte; MHC; Superantigen; T cell receptor; Molecular biology

1. Introduction

T-cell superantigens work by binding to MHC class II, then activating T-cells by recognizing the Vβ portion of the T-cell receptor (TCR) (Scherer et al., 1993; Webb and Gascoigne, 1994; Gascoigne et al., 1995). They are produced as part of the life cycle of certain infectious agents, where they act to influence the immune system to the advantage of the parasite. Superantigens appear to act as virulence factors in *Staphylococcus aureus* (Rott and Fleischer, 1994) and are necessary to the life cycle of Mouse mammary tumor viruses (MMTV) (Golovkina et al., 1992; Held et al., 1993). In addition to defined superantigens produced by MMTV, *Staphylococcus*, *Streptomyces*, *Mycoplasma* and *Yersinia*, undefined superantigens are implicated in a number of diseases including tuberculosis (Ohmen et al., 1994), diabetes (Conrad et al., 1994), toxoplasmosis (Denkers et al., 1994) and cytomegalovirus (Dobrescu et al., 1995). It would be useful to be able to clone genes expressing potential superantigens from these organisms and disease states on the basis of their superantigenic properties. Currently, this has not been accomplished and all the known superantigens have been cloned by classical techniques, independent of the special properties of a superantigen.

Expression of proteins on the surface of bacteriophage ('phage display') allows selection of phage

Abbreviations: TCR, T-cell receptor; MHC, major histocompatibility complex; Vβ, variable region domain of the TCR β-chain; gIIIp or gVIIIp, proteins encoded by gene 3 or 8 of filamentous bacteriophage; SEA; staphylococcal enterotoxin A

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particles on the basis of binding to an appropriate ligand (Scott and Smith, 1990; Smith, 1991; Barbas, 1993). Cloned genes are expressed as part of the phage coat proteins; as fusions with the gene III protein (gIIIp) (Scott and Smith, 1990; Lowman et al., 1991) or the gene VIII protein (gVIIIp) (Chang et al., 1991; Kang et al., 1991). Because each phage particle contains the gene encoding the displayed gene product, this method allows genes to be cloned on the basis of the protein's binding properties. Sequential rounds of panning on ligand bound to a solid phase selects those phage particles that express surface proteins interacting with the ligand. Enrichment factors of 10^6–10^7-fold are possible. The main advantage of this technique is that the DNA encoding the selected protein product is selected at the same time. We are interested in the potential of phage display libraries to clone novel superantigens or to produce mutant superantigens with novel properties. As a first step, it is necessary to demonstrate that a superantigen expressed on the surface of a phage particle is capable of interacting with MHC class II and TCR proteins. We have therefore expressed the staphylococcal enterotoxin A (SEA) protein on phage surfaces as a fusion with the gVIII protein. We demonstrate that in this form it binds to anti-SEA antibodies and to MHC class II, and that it activates T-cells in a Vβ-specific manner. In addition, we have developed a simple method for selecting phage that bind to proteins expressed on cell surfaces. This will be particularly useful in selecting phage that bind to cell surface molecules that are difficult to purify for use in the standard plastic micropanning protocols.

2. Materials and methods

2.1. SEA-phage construction

The filamentous phage vector f88-4 was provided by Dr. George Smith (University of Missouri, Columbia, MO). This forms a fusion protein between the C terminal of the inserted gene's product and the N terminal of gVIIIp (Zhong et al., 1994). SEA clone pKH-X35 was obtained from Dr. John Fraser (Univ. of Aukland, New Zealand). PCR with Vent Polymerase (NEB) was used to mutate the 5′- and 3′-ends of the SEA gene for cloning into f88-4. The construct is shown in Fig. 1. The 5′ oligonucleotide used was 5′-CTCCAAGCTTTGCCAGC-GAGAAAAGCGAAG-3′. Two 3′ oligonucleotide primers were used. For the construct with the five amino acid linker between SEA and gVIIIp (SEA-L) (Fig. 1b) the primer 5′-GCCTCCTGCAGATCCAC-CGCCTCCGGATGT-ATATAAATATATATC-3′, and for the non-linker version (SEA-P) (Fig. 1c); 5′-GCCTCCTGCAGATGTATATAAATATATATC-3′. The two SEA PCR products were cut with *Hind*III and *Pst*I and cloned into f88-4. They were trans-

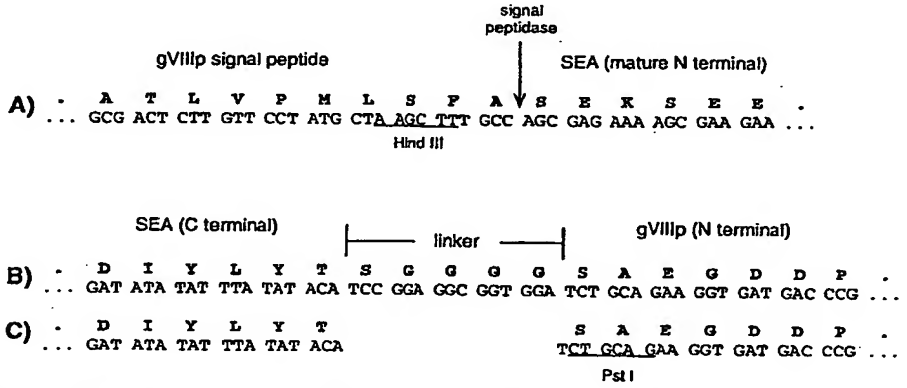

Fig. 1. SEA-phage fusion protein constructs for phage vector f88-4. (*a*) The N-terminal of the gVIIIp fusion protein is shown. The signal peptidase should cleave the protein to leave the authentic SEA N-terminus. (*b*, *c*) The linkage between the C-terminus of the SEA sequence and the gVIII protein in f88-4, showing the linker present in the SEA-L construct (*b*).

formed by electroporation into *E. coli* strain DH5α and sequenced.

Phagé were produced by growing the transformed bacteria overnight in 0.5 l of L broth with 20 μg/ml tetracycline. The culture was spun down twice (8000 × *g* for 15 min) and the phage were precipitated out of the cleared supernatant by the addition of 0.15 vols. of PEG/NaCl solution (17% PEG 8000, 19.5% NaCl in water). After incubation at 4°C for 2 h, the phage were spun down (8000 × *g* for 40 min at 4°C), resuspended in TBS and sterile-filtered through a 0.22-μm membrane. There is a potential risk of infection of other bacteria expressing pili with the SEA-bearing phage. Recombinant phage were therefore handled using NIH/CDC Biosafety level 2 procedures.

2.2. Antibodies

Anti-SEA mAb ECA1 (Lapeyre et al., 1987), and rabbit anti-SEA serum were a gift of Dr. S. Kaveri (Institute Pasteur, Paris). Anti-Thy-1 and anti-TCR Vβ reagents were obtained from Pharmingen (San Diego, CA). Anti MHC class II reagents B7/21 (anti-DP), aLeu10 (anti-DQ), and L243 (anti-DRα) (Robbins et al., 1987) were gifts of Drs. F. Brodsky (Univ. of California, San Francisco). DA6-147 (anti-DRα) and TAL14.4 (anti-DRβ) were from Dr. P. Travers, (Univ. of London, UK), and Q5/13 (anti-DRβ) was from Dr. V. Quaranta (TSRI). Purified mouse serum IgG was obtained from Sigma (St. Louis, MO)

2.3. Phage selection by micropanning

Binding to antibody was assessed by attaching mAb ECA1 to the surface of 96-well ELISA plates, as described (Gascoigne and Ames, 1991), blocking with 1% BSA, incubating with 100 μg/ml of SEA (Toxin Tech) or PBS as a control, and then incubating with the various phage preparations for > 2 h at 4°C. The phage were then eluted with 0.1 M HCl pH 2 (adjusted with glycine) for 10 min, neutralized and used to infect starved *Escherichia coli* MC1061 F' Kan. The infected bacteria were then spread on tetracycline (20 μg/ml) LB agar plates. After overnight culture tetracycline-resistant colonies were counted, representing the number of transducing units (TU) recovered. To determine the number of SEA-bearing phage among the tetracycline-resistant colonies, colony blotting was performed by standard techniques, probing with a ^{32}P-labeled SEA probe (Sambrook et al., 1989). In some experiments, an antibody-based variant of this technique was used, probing with a rabbit anti-SEA serum as for a Western blot as described (Wung and Gascoigne, 1996) (below).

2.4. Phage selection by cell binding

Phage were selected for binding to cells as follows. Cells were washed in PBS and resuspended at $1-2 \times 10^7$/ml in PBS. Phage preparations were added to 100 μl of cells in 15-ml tubes. Usually, 10^8-10^{10} phage were added to the cells and incubated at room temperature for 1–3 h. The cells were washed in PBS three times and resuspended in 50 ml of pH 2 elution buffer for 10 min. The cells were then spun down, and the eluate removed to an Eppendorf tube. The cleared eluate was then neutralized and the number of recovered TU and/or SEA-bearing phage was assessed as before. When blocking agents, such as natural SEA or antibodies to the cell-surface molecules, were tested, these were added to the cells 30 min prior to the addition of the phage. The cells used were Raji; a Burkitt's lymphoma cell expressing large amounts of HLA-DP, DQ and DR which binds strongly to SEA (Fischer et al., 1989), and RM3, a class II-negative variant of Raji (Calman and Peterlin, 1987).

2.5. FACS analysis of activated T-cells

Splenocytes were obtained from B10.BR mice and a single-cell suspension was prepared. The splenocytes were resuspended in RPMI supplemented with 10% FCS, 1% glutamine, Pen/Strep and 0.5 mM 2-mercaptoethanol. The cells were then plated into 96-well plates at 1×10^6 cells/ml and phage, SEA and ConA were added at various dilutions. After 48 h, IL-2 was added at 100 U/ml. The cells were prepared for FACS analysis on day 6 by staining with anti-Thy1.2-FITC, anti-Vβ3-PE and anti-Vβ6-PE (Pharmingen, San Diego, CA). Flow cytometry was performed on a Becton–Dickinson FACScan (Mountain View, CA).

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2.6. Western blotting

Phage and controls were electrophoresed on a 12.5% SDS–PAGE gel. They were then transferred by standard Western blot technique onto a nitrocellulose membrane (Schleicher and Schuell). The membrane was blocked with 10% instant non-fat dried milk (Vons, Los Angeles) in TBS and incubated with a purified rabbit anti-SEA antiserum (2 μg/ml). The membrane was washed with 0.1% Tween–TBS and incubated with a horseradish peroxidase-linked donkey anti-rabbit Ig antibody (Amersham). The bound antibodies were then detected with an ECL chemiluminescence detection kit (Amersham).

Fig. 2. Western blot of wild-type and recombinant SEA-phage, and natural SEA. The blot was probed with purified rabbit anti-SEA serum followed by horseradish peroxidase-conjugated donkey anti-rabbit serum.

3. Results

3.1. Production of SEA-bearing phage

Vector f88-4 has two copies of the gVIII protein; one wild-type copy and one with *Hin*dIII/*Pst*I cloning sites at the amino terminus of the mature protein (Zhong et al., 1994). Since both the wild-type and fusion proteins should be expressed on the phage surface, the recombinant phage retain full infectivity. Although the fusion protein is potentially expressed in a multivalent array on the phage surface, experiments with antibodies expressed in this system indicate that in practice only a single copy of such a relatively large protein is expressed (A. Kang, TSRI, personal communication). Insertion of a recombinant sequence after the signal peptidase site will result in a fusion protein with a free amino terminus and the carboxyl terminus linked to the gVIII protein attached to the phage surface. Fig. 1a shows the N terminal of the SEA–gVIIIp fusion protein. Two versions of the SEA fusion protein were made, differing at the C terminal fusion to gVIIIp. Fig. 1b shows a version with a 5-amino acid linker sequence (SGGGG) on the C terminus before the gene VIII sequence. This construct is designated SEA-L. The SEA-phage construct without this linker (designated SEA-P) was also produced (Fig. 1c). The flexible linker peptide was added to attempt to reduce steric hindrance of SEA–ligand interactions by the rest of the gVIIIp molecule.

To confirm that the recombinant SEA-phage were expressing SEA, 1 ng of purified SEA and 1.5 μg of the wild-type, SEA-phage (SEA-P) and SEA–linker phage (SEA-L) were electrophoresed on SDS–PAGE and immunoblotted. The blot was developed with a polyclonal anti-SEA antibody (Fig. 2). Several protein bands were present in the SEA-phage lanes that are were also present in the wild-type (wt) phage lane. Two bands were visible in the SEA-phage lanes that were not present in the wt phage. These migrated near the predicted molecular weight of the fusion protein, ~35 kDa. The larger of these two bands was more intense than the smaller, particularly in SEA-L. In addition, this band was slightly larger in the SEA-L than in the SEA-P, indicating that this was the fusion protein and that the slight increase in size corresponded to the 5 extra amino acids in this species. This presumed SEA–gVIIIp band was substantially stronger in the SEA-L than in the SEA-P suggesting either that a larger quantity of it is expressed or that it is more readily recognized by the antiserum.

3.2. Selection of SEA-phage by antibody

The functional expression of SEA by the recombinant phage was assessed by micropanning. Monoclonal anti-SEA antibody (ECA1) (Lapeyre et al., 1987), BSA, or control antibodies were bound to wells of a 96-well plate as described (Gascoigne and

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Ames, 1991). After washing and blocking with BSA, similar amounts of the wild-type and SEA-phage were incubated in the wells. The wells were washed and then eluted at pH 2. The eluate was neutralized and used to infect starved MC1061 F′ Kan cells. Colonies growing on tetracycline plates were counted and used to calculate the number of transducing units (TU) in the starting eluate. We found that the SEA-L specifically bound to the anti-SEA antibody (Fig. 3a). This binding was inhibited by the presence of SEA (100 μg/ml). Binding of the SEA-P to ECA1 was similar to wt phage in this experiment. This suggests that the lack of a linker between SEA and gVIIIp in the SEA-P protein may interfere with the ECA1 binding site on SEA.

The ECA1 antibody was also able to select for the SEA-phage from a mixture with wt phage. A mixture of wt phage and either SEA-P (2.5%) or SEA-L (1.4%) was incubated in the antibody-coated wells. After washing and eluting, the eluates were used to infect MC1061 F′ Kan and plated out. The resulting colonies were then screened by DNA hybridization to determine the proportion of colonies that contained the SEA gene (Fig. 3b). The colonies derived from the eluate from the ECA1-incubated phage preparations were 26% positive for the SEA-P and 34% positive for the SEA-L. These were about 10-fold and 25-fold enrichments, respectively. Therefore the anti-SEA selects SEA-bearing phage from a mixture in which it is a small minority. The absence of a linker in the SEA-P did not seem to strongly affect the specificity of the selection. In other experiments, we have found > 70% of SEA-P colonies positive after selection from a similar mixture of wt and recombinant phage (not shown). Thus, despite the low number of SEA-P recovered (Fig. 3a), they are strongly selected for expression of the SEA protein. An anti-TCR mAb did not increase recovery of SEA-phage nor select them from mixtures with wt (not shown).

3.3. SEA-phage bind to MHC class II-bearing cells

To assess the ability of MHC class II proteins to bind and select the phage, we used a cell-binding assay rather than the 'micropanning' assay. Raji or RM3 cells were incubated with the phage. Raji cells express high levels of MHC class II molecules and bind staphylococcal enterotoxins very well (Fischer et al., 1989), whereas RM3 is a class II-negative variant of Raji (Calman and Peterlin, 1987). After washing, the bound phage were eluted at pH 2 and

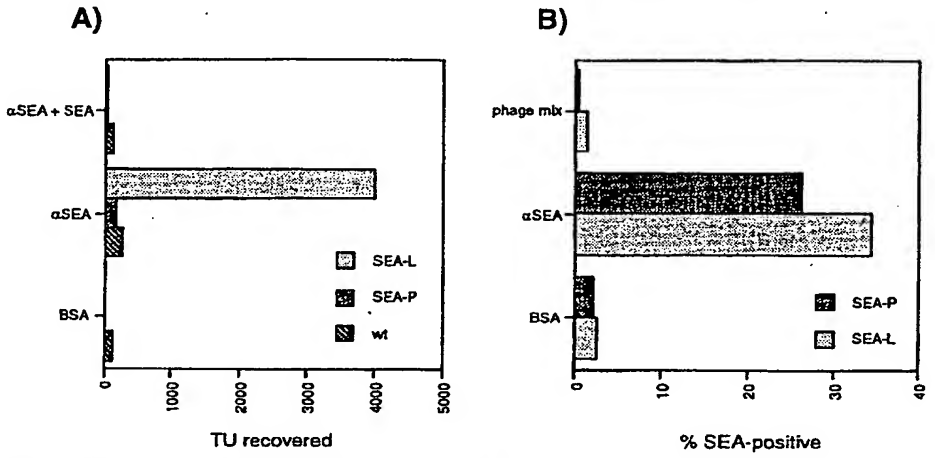

Fig. 3. SEA on phage surfaces binds anti-SEA mAb (*a*) and can be selected by anti-SEA (*b*). Anti-SEA mAb ECA1 (Lapeyre et al., 1987) or BSA (50 μg/ml) were bound to microtiter wells. About 10^8 TU of phage were incubated in the wells. After washing and eluting, the phage were used to infect bacteria. (*a*) SEA-L bound to anti-SEA and this binding was inhibited by natural purified SEA (100 μg/ml). SEA-P bound poorly to the mAb. (*b*) Anti-SEA selected both SEA-P and SEA-L out of a ~ 100-fold excess of wild-type phage. Colonies were counted and blotted onto nitrocellulose, followed by hybridization with anti-SEA antiserum as in Fig. 2.

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were used to infect bacteria. The number of recovered TU was determined as before. The SEA-L were able to bind MHC class II-bearing Raji cells but not class II-negative RM3 cells (Fig. 4a). The binding of the SEA-P was very poor. Binding of SEA-L to Raji cells was completely inhibited by SEA (100 mg/ml).

Raji cells were then used to select for the SEA-bearing phage. A 1/26 mixture of SEA-P and SEA-L with wt phage was incubated with Raji or RM3 cells. The TU recovered from the Raji cells were 43% SEA-positive from the SEA-P mix and 33% SEA-positive from the SEA-L mix (Fig. 4b), an enrichment of about 10-fold for each. RM3 did not select for SEA-positive phage. In other experiments using SEA-P, better enrichment was obtained. For example, from a 1/250 mixture of SEA-P and wt phage, 65% of the resulting TU were SEA-positive, a 160-fold enrichment. In no case did the RM3 cells select SEA-phage (data not shown).

A panel of anti-MHC class II antibodies were used to inhibit the binding of the SEA-L to Raji cells (Fig. 5). Raji cells were incubated with 50 μg/ml each of B7/21 (anti-DP), aLeu10 (anti-DQ), DA6-147 (anti-DRα), L243 (anti-DRα), Q5/13 (anti-DRβ) and TAL14.4 (anti-DRβ) (Robbins et al., 1987). When SEA-L were incubated and eluted from the Raji cells as before, 50-fold fewer TU were recovered than from control Raji cells than had been incubated with purified mouse IgG (0.3 mg/ml). Thus, binding to Raji cells was inhibited by a cock-

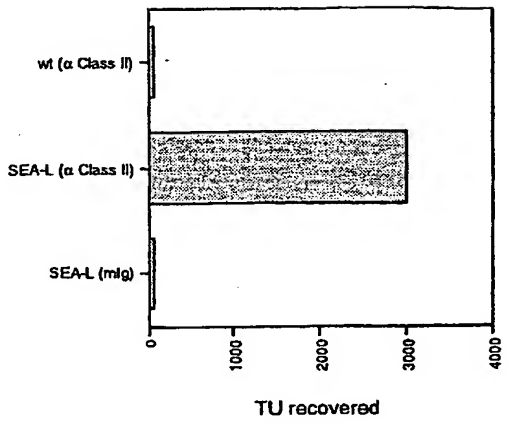

Fig. 5. Raji cells were incubated for 30 min with a cocktail of anti-class II mAbs or with an equivalent concentration of irrelevent mouse IgG. The cells were then mixed with SEA-L or wild-type phage for 2 h, followed by washing, elution and infection. The number of TU recovered was calculated as before. For comparison, the recovery of wt phage was normalized to the input of wt phage compared to SEA-L.

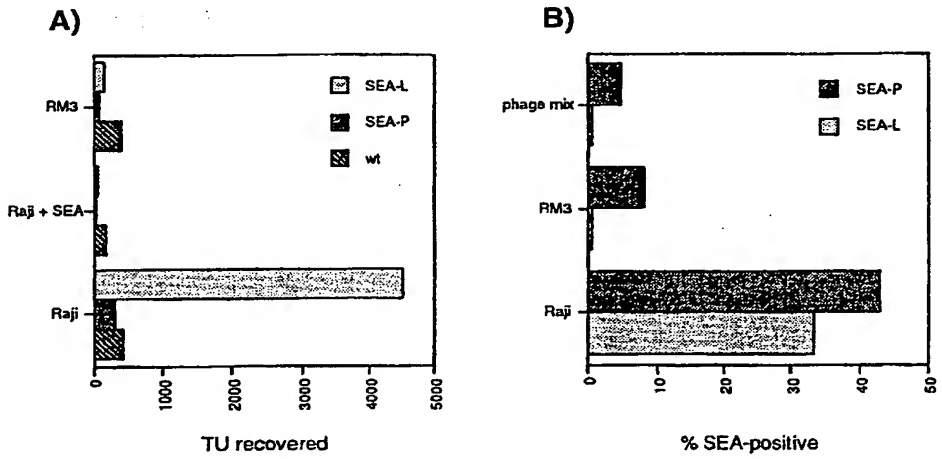

Fig. 4. SEA-phage bind to class II-expressing cells. (*a*) SEA or wild-type phage were allowed to bind to class II-positive Raji cells in the presence or absence of 100 μg/ml of natural SEA. Alternatively, they were allowed to bind to the class II-negative variant of Raji, RM3. After washing and elution, they were used to infect bacteria and the number of TU recovered was calculated. SEA-L bound to Raji, but not RM3, and was inhibited by natural SEA. (*b*) A similar experiment was performed with SEA-P or SEA-L mixed with wild-type phage. The colonies recovered were screened by colony blotting and hybridization with a ^{32}P-labeled SEA probe.

tail of anti-class II reagents. Because Raji cells express large quantities of different class II molecules of different haplotypes, we were unable to block SEA-phage binding with individual mAbs (data not shown).

3.4. Interaction between SEA-phage and TCR

We attempted to bind SEA-phage to T-cells as for the class II-expressing cells. Although we found evidence for binding and selection in some experiments, we were unable to show reproducible specific binding to TCR-positive cells (data not shown). This is similar to our experience with labeled natural SEA (C. Haarstad and N.R.J. Gascoigne, unpublished; see Gascoigne et al., 1995), and is predictable, given the fast off-rate for the enterotoxin–TCR interaction (Seth et al., 1994; Malchiodi et al., 1995). As an alternative method, we used the SEA-phage to try to activate T-cells in vitro and in vivo. B10.BR splenocytes were cultured in vitro with Con A, SEA-P, SEA-L, wild-type phage or natural SEA for 6 days. The cells were stained for Thy-1 and various Vβ elements. The T-cells incubated with SEA or with the SEA-bearing phage preparations showed an increase in the number of $V\beta3^+$ T-cells compared to those incubated with wt phage or ConA (Table 1). Cells stimulated with the SEA-L showed an increase in $V\beta3^+$ cells (12% compared to 2% $V\beta3^+$ in wt phage and 6% in ConA-stimulated cells). Cells stimulated with the SEA-P also showed an increased number of $V\beta3^+$ cells. Cells stained with an anti-Vβ6 control antibody showed decreased numbers of $V\beta6^+$ cells in the SEA, SEA-P and SEA-L, presumably corresponding to the increase in $V\beta3^+$ cells. Similar results were also obtained from in vivo experiments, where the SEA-phage were injected into the hind footpads of mice (data not shown).

Table 1
SEA-phage stimulate $V\beta3^+$ T-cells

Activation	$\%V\beta3^+$	$\%V\beta6^+$
ConA	5.7	15.9
SEA	48.2	5.2
wt phage	2.0	9.5
SEA-L	12.3	4.5
SEA-P	7.3	7.9

Phages were added to B10.BR spleen cells, which were cultured for 2 days, at which point IL-2 was added (100 U/ml). After a total of 6 days, the cells were analyzed by FACS. The percentage of $V\beta3^+$ and $V\beta6^+$ cells among the Thy-1^+ cells was determined.

4. Discussion

We have demonstrated that the superantigen SEA can be expressed on the surface of bacteriophage as a fusion with the gVIII protein. It is reactive with antibodies to SEA and binds to MHC class II molecules. The SEA–gVIIIp phage can activate T-cells in a Vβ-specific manner. Thus the phage-displayed SEA retains the MHC class II and TCR Vβ-specificity that define a superantigen.

Two different SEA–gVIIIp constructs were tested. One, denoted SEA-P, consists of the SEA sandwiched between the signal peptide and the mature protein sequence of gVIIIp, so that the C-terminal of SEA abuts the N-terminus of gVIIIp. The second construct, SEA-L, includes a 5-amino acid flexible linker sequence between the SEA C-terminus and the gVIIIp N-terminus. Both constructs produced SEA detectable by Western blotting with rabbit anti-SEA polyclonal antiserum. However, the SEA-L contained more detectable chimeric protein. In plate binding experiments with a monoclonal anti-SEA reagent, the SEA-L construct bound much better than the SEA-P construct. Similarly, the SEA-L construct was much stronger in binding to MHC class II. The crystal structure of SEA has recently been determined (Schad et al., 1995). The C-terminus is shorter than the corresponding region of SEB (Swaminathan et al., 1992), so that a protein attached directly to the C-terminal is more likely to cause steric interference between the two proteins, since the SEB C-terminus extrudes further out of domain 2. The SEA C-terminus is on the opposite face of domain 2 from the Zn^{2+}-binding site that is involved in the interaction with the β-chain α-helix of MHC class II and is closer to the TCR interaction site (Irwin et al., 1992). Thus the linker in the SEA-L construct probably increases the separation between the two parts of the fusion protein, allowing the superantigen to function more effectively.

The phage-displayed SEA bound readily to MHC class II expressed on Raji cells. It did not bind to the

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class II-negative variant of Raji, RM3 (Calman and Peterlin, 1987), indicating that binding to the cell surfaces was class II-specific. This was confirmed by blocking experiments showing that a mixture of anti-class II mAbs blocked binding to the Raji cells. Binding of SEA-L to class II was inhibited very effectively by natural SEA.

We found that both anti-SEA and MHC class II-bearing cells were able to select SEA-bearing phage out of mixtures with wt phage. Interestingly, although the SEA-L construct gave higher numbers of recovered phage particles than the SEA-P construct (Fig. 3a, Fig. 4a), the presence of the linker did not improve the selectivity (Fig. 3b, Fig. 4b).

SEA is known to interact with mouse Vβ3 TCRs (Takimoto et al., 1990; Gascoigne and Ames, 1991; Irwin et al., 1992). In order to demonstrate this interaction with the SEA-phage, we first attempted cell-binding assays as performed with the class II^+ cells above. We were unable reproducibly to demonstrate binding of SEA-P or SEA-L to cells bearing Vβ3 TCR. This is similar to results with binding of labeled superantigens to T-cells (Gascoigne et al., 1995). This interaction could not be directly demonstrated, probably because of the comparatively low number of TCRs present on the cell surface, and because of the fast off-rate (0.01 to $> 0.1\ s^{-1}$) of the interaction between TCR and superantigens (Seth et al., 1994; Malchiodi et al., 1995). The superantigenic capability of the phage-expressed SEA was demonstrated by T-cell activation. SEA-bearing phage preparations were able to stimulate expansion of $V\beta3^+$ populations in culture.

Our data show that cell-surface ligands, like plastic-immobilized ligands (Scott and Smith, 1990; Lowman et al., 1991; Kang et al., 1991), can be used to 'pan' for phage-expressed proteins out of mixtures of other phages. This method has previously been used to select for antibodies to blood group antigens (Marks et al., 1993; Siegel and Silberstein, 1994) and for lymphocyte cell-surface antigens (de Kruif et al., 1995), using erythrocytes and peripheral blood leukocytes, respectively. This study extends this method to other types of receptor–ligand interaction. Many cell-surface receptors and ligands are multimers, often consisting of different polypeptide chains. This should, therefore, be a valuable addition to methods for selecting ligands to cell-surface proteins, since these are frequently difficult to purify or to express. In the case studied here, an MHC class II-positive and negative variant of the same cell were available. This has obvious advantages in terms of defining the specificity of the selection, although it is probably not an absolute requirement for the use of cell panning.

The ability to express superantigens on bacteriophage, where the gene is linked to the gene product, will allow us to develop cloning strategies for other superantigens using cDNA libraries expressed in phage-display systems (Crameri and Suter, 1993). Several infectious agents appear to express a superantigen, including *Mycobacterium tuberculosis*, *Toxoplasma* and cytomegalovirus (Ohmen et al., 1994; Denkers et al., 1994; Dobrescu et al., 1995). A superantigen has also been implicated in diabetes (Conrad et al., 1994). In addition, the ability to generate libraries of mutants in phage will allow us to select for superantigens with novel specificities or properties.

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